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Original Article

Lin28 mediates chemoresistance through inhibition of apoptotic cell death in gastric carcinoma cells

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Abstract: Cumulative data from clinical trials and retrospective analyses suggest that Lin28 contributes to chemoresistance in patients with gastric carcinoma. However, there is no information available to show the mechanism of action of Lin28, and its molecular targets are not completely understood. The effect of Lin28 on cell chemotherapy resistance was determined in gastric carcinoma cells after enforced Lin28 overexpression or small interfering RNA knockdown. The effect of Lin28 in regulating IκBα and Let-7 miRNA was also evaluated. Expression of Lin28 was evaluated by immunohistochemical scoring on tumor tissues obtained before and after neoadjuvant chemotherapy. The pathologic response of gastric carcinoma was graded according to Sataloff's classification. To investigate the possible influence of Lin28 on the therapeutic efficacy of cisplatin and its underlying mechanism, we established several Lin28 positive cell lines by stable transfection of Lin28 expression vectors into SGC7901 cells. The overall cytotoxicity of cisplatin was significantly reduces in SGC7901 cells with Lin28 expression compare to parental SGC7901 cells. Further analyses indicate that expression of Lin28 in SGC7901 cells mainly interferes with cisplatin-induced apoptotic cell death. Moreover, further studies showed that IκBα and Let-7 miRNA were the molecular targets of Lin28. Overexpression of Lin28 in gastric carcinoma cells considerably inhibited IκBα and Let-7 miRNA levels. In clinical trials, we found that Lin28 expression in gastric carcinoma was correlated with tumor regression grade (P=0.024), and higher expression of Lin28 was more frequently found in nonresponding patients. The results obtained from this study provide useful information for understanding Lin28 mediated resistance to cisplatin. The future investigation on the precise roles of Lin28 in gastric carcinoma will provide new insights, which will contribute to improve diagnosis and treatment.

Keywords: Gastric carcinoma, Lin28, chemotherapy, Let-7

Introduction

Currently gastric carcinoma was one of the most common cancers worldwide. In spite of recent improvements in early diagnosis and treatment, gastric carcinoma is one of the leading causes of cancer related death in China [1]. Until now radical surgery is the only chance for cure, however, a large amount of carcinomas are diagnosed at an advanced or recurrent stage. A curative resection could be performed the overall survival was about 45% after 5 years in advanced gastric cancer [2]. Other means to improve survival is adjuvant chemotherapy. The patients with advanced or recurrent gastric cancer remains poor prognosis and these patients should be treated with chemotherapy [3, 4]. However, a significant proportion of gastric carcinoma cells are resistant to chemotherapeutic agents. As a result, chemotherapy resistance has become the major cause of cancer chemotherapy failure and is largely responsible for gastric carcinoma mortality. Therefore, the identification of patient-specific tumor characteristics that can improve the ability to predict response to therapy would help optimize treatment, improve treatment outcomes, and avoid unnecessary exposure to potential toxicities.

Lin28 is a highly conserved RNA-binding protein, it consists of a cold shock domain and

retroviral-type (CCHC) zinc finger motifs [5]. Lin28 is readily expressed in embryos and embryonic stem cells, but it is either undetectable or its expression remains at low levels in normal adult tissues, suggesting that Lin28 may plays a critical role in cell proliferation and/or differentiation during embryonic development. Recently, Lin28 was confirmed ability to facilitate the reprogramming of human somatic cells to induced pluripotent stem (iPS) cells [6]. Thus Lin28 play an important role in stem cells, and the expression of Lin28 carcinoma cells may have stem cell-like features. Moreover, expression of Lin28 in tumor cell lines showed resistance to apoptosis and chemotherapy in tumors [7].

Lin28 was expression in various human epithelial-type neoplasias, such as breast cancer, lung cancer, ovarian cancer, hepatocellular cancer and colorectal cancer [7-10]. High expression of Lin28 is a poor prognostic factor in gastric carcinoma, and it is significantly associated with lymph node metastasis in colorectal cancer [10, 11]. Recently, Lin28 was confirmed ability to mediate paclitaxel resistance in breast carcinoma [7]. Our present data show that Lin28 expression was an independent predictive factor for pathologic response to neoadjuvant chemotherapy in gastric carcinoma [12]. However, there is no information available to show the mechanism of action of Lin28 and its molecular targets are not completely understood. This prompted us to evaluate Lin28 status in gastric carcinoma cells.

Although Lin28 has been extensively studied in other cancers, no investigation has been conducted in gastric carcinoma. We hypothesize that Lin28 may play a role in mediating chemoresistance of gastric carcinoma. To investigate the underlying mechanisms, we analyzed the role of Lin28 in the regulation of IκBα, bcl-2, cyclin B1, Akt and Let-7 miRNA. The present study suggests that Lin28 may be a potential target to overcome chemoresistance and provides a scientific basis for further investigation of mechanisms of chemoresistance.

Methods

Cell culture

The human gastric cancer cell lines (MKN28, MKN45, SGC7901, SGC823) were obtained from American Type Culture Collection (ATCC, Manassas, VA) and cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum, 100 units/mL penicillin, and 100 mg/mL streptomycin (Invitrogen, Carlsbad, CA). In all of the experiments, cells were grown at 37°C

Western blot analysis

Cellular proteins were isolated using a protein extraction buffer containing 150 mmol/L NaCl, 10 mmol/L Tris (pH, 7.2), 5 mmol/L ethylenediaminetetraacetic acid, 0.1% Triton X-100, 5% glycerol, and 1% sodium dodecyl sulfate. Equal amounts (40 lg/lane) of proteins were fractionated on 10% sodium dodecyl sulfate polyacrylamide gels and transferred to polyvinylidene difluoride membranes. After incubation with anti-Lin28 or GAPDH (Santa Cruz Biotechnology) antibody for 2 h at room temperature and a subsequent wash with TBS-Tween 20 (0.1%), the membranes were incubated with peroxidase-conjugated rabbit anti-mouse secondary antibody (Santa Cruz Biotechnology) for 2 h at room temperature and subjected to enhance chemiluminescent staining using an ECL detection system (Bio-Rad, Hercules, CA). Experiments were repeated at least three times.

Methyl-thiazolyl-tetrazolium (MTT) assay

Cell viability was measured using an MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] assay (Amresco, Solon, OH) according to the manufacturer's protocol. Cells were seeded in 96-well plates at a density of 1×10^3 cells per well. After treatment, the cells were incubated with 5 mg/ml MTT for 4 h. Then, the medium was removed and 150 ml of sterilized DMSO solution was added, followed by incubation at 37°C for 4 h. The absorbance of the reaction solution at 570 nm was measured, and these data were used to make growth curves.

Flow cytometric analysis

After culture and treatment with cisplatin in six-well plates for 48 h, the SGC7901 with or without expression of Lin28 were collected, fixed in 70% ethanol at 20°C overnight, washed twice in PBS, and incubated in 50 μg/mL of RNase for 10 min at 37°C. Before analysis, cells were stained with 50 μg/mL of PI at room tempera-

ture for 30 min. Analyses were performed using a FACScan flow cytometer (Becton Dickinson, Sunnyvale, CA).

Cytospin preparation

After designated treatments, SGC7901 cells with or without expression of Lin28 were harvested by trypsinization and washed with PBS. Approximately 1×10^5 cells were plated onto microscope slides using the Cytospin 3 cell preparation system. Slides were air dried and fixed in absolute methanol before Giemsa staining. Slides from three independent experiments were examined and photographed using bright-field microscopy.

RNA extraction and cDNA synthesis

Total RNA was extracted from gastric carcinoma cells using the Trizol reagent (Invitrogen life Technologies, USA). The isolated RNA was reverse transcribed using M-MLV reverse transcriptase. Briefly, the RNA was denatured by heating for 5 min at 70°C, cooled on ice, and incubated with reverse transcriptase reaction mixture. The standard mixture contained 2 μg of total RNA, 25 U of RNAse inhibitor, 0.5 mM each of dNTPs, 1.5 μM reverse primer and 200 U of M-MLV reverse transcriptase in a total volume of 25 μl. For reverse transcription, tubes were incubated at 42°C for 60 min, followed by rapid cooling.

Real-time quantitative PCR

Real-time RT-PCR analyses were performed with the ABI Prism 7500 sequence detection system (Applied Biosystems, Foster City, CA). Briefly, 25 μl reaction mixture containing 2 μl of cDNA template, 1 μl each of sense and antisense primers, 1× SYBR Green Universal PCR Mix was amplified as follow: denaturation at 95°C for 5 min and 30 cycles at 95°C for 30 s, 58°C for 30 s and 72°C for 40 s. Real-time quantitative PCR reaction was performed in triplicate for each sample and a mean value used to calculate mRNA levels. Quantitative analysis was performed using the comparative CT method [13, 14]. The Lin28 copy numbers in normal and tumor tissues were normalized to mRNA copy numbers of the house keeping gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) to give a value ΔCT. This final value was to determine changes in expression of Lin28 in each sample. The primer sequences for Lin28 were as follows: forward primer 5'-ACCGGACCTGGTGGAGTATTC-3', reverse primer 5'-GGTAGGGCTGTGGATTTCTTC-3'. The primers for GAPDH were taken from a previously published assay [13]. Fluorescent data were converted into RQ measurements, which stand for relative expression automatically by the SDS system software and exported to Microsoft Excel. Thermal dissociation plots were examined for biphasic melting curves, indicative of whether primer dimers or other nonspecific products could be contributing to the amplification signal.

Human stomach tissue specimens

Twenty-one consecutive gastric carcinoma patients with informed consent under the guideline of Ethics Committee were obtained presented at the Zhejiang University Sir Run Run Shaw Hospital, who received neoadjuvant chemotherapy during the period of February 2004 to January 2010, were enrolled in this study. Patients with neoadjuvant chemotherapy before surgery, and those whose paraffin-embedded tissue from the gastroscopy was insufficient to allow a pathologic diagnosis and evaluation of biomarkers, were excluded. In case of partial response, two more courses were given, or the patients underwent operation soon. The ethical committee of Sir Run Run Shaw Hospital approved this study. Informed consents from patients were obtained for the use of paraffin-embedded tumor tissues.

The pathologic features of the specimens were classified based on the 7th edition of the International Union against Cancer and the American Joint Committee on Cancer. Prior to the analysis, gastroscopy and resected specimens were fixed with 10% formaldehyde, embedded in paraffin blocks, cut into 4-μm-thick sections, and mounted onto glass slides.

Assessment of pathologic response

Tumor tissues were obtained 2-3 days prior to chemotherapy via core biopsy or during the surgery. Tumor samples obtained before and after chemotherapy was fixed with 10% buffered-formalin, embedded in paraffin and stained with hematoxylin and eosin. The pathologic response of gastric carcinomas was graded according to Sataloff's classification (primary

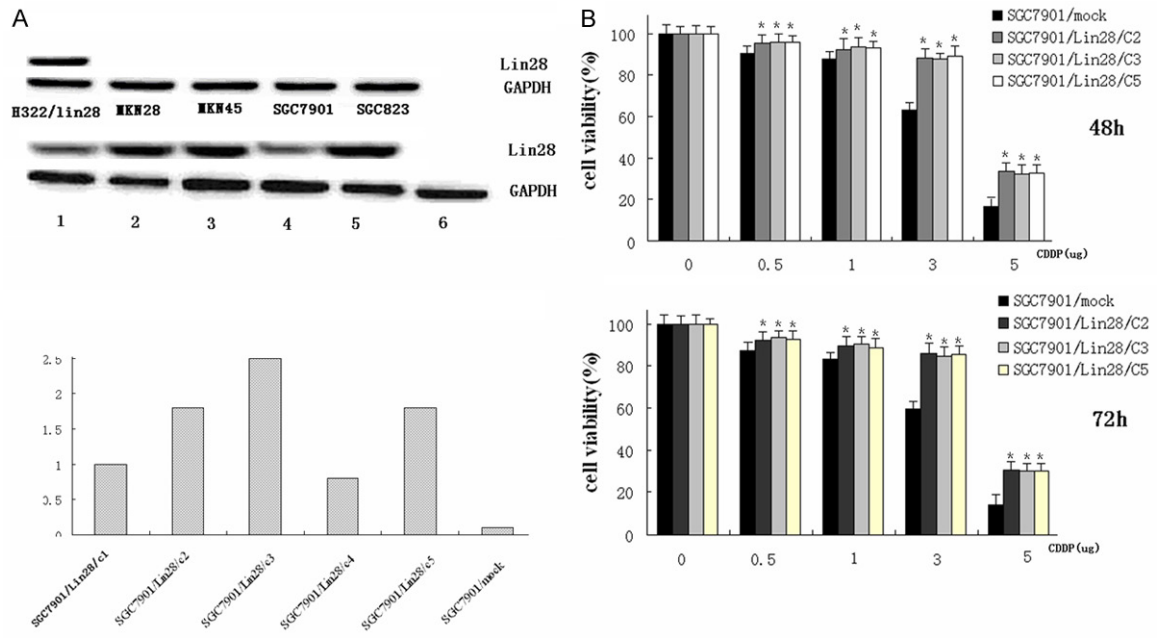

Figure 1. Lin28 expression attenuates the overall cytotoxicity of cisplatin in gastric carcinoma cells. A. Lin28 expression was determined in the indicated cancer cell lines and their chemoresistant subclones by Western blotting. GAPDH expression was used as a loading control, and the stable H322/Lin28 lung cancer cell line was used as positive control for Lin28 expression as described in Materials and Methods. Five individual Lin28-transfected clones 2, 3 and 5 (lanes 2, 3 and 5, SGC7901/Lin28/c2, SGC7901/Lin28/c3 and SGC7901/Lin28/c5), express high levels of exogenous Lin28. Clone 1 and 4 express low levels of exogenous Lin28 protein (lane 1 and 4). SGC7901 cell line transfected with empty vector does not express Lin28 (lane 6, SGC7901/mock). B. MTT assays. Cells cultured in 96-well and viability was evaluated by MTT assays after both 48 and 72 h of cisplatin treatment. Cells treated with PBS were used as a control. Columns, mean of three independent experiments; *P < 0.001, when compared with the group treated with cisplatin in the SGC7901/mock.

site response classification) [15]. Given the nature of their results and using the same guidelines, we re-named the four categories in Sataloff's classification into two groups: Group I as responders (major pathologic response) and Group II as nonresponders (minor or no response), both of which contain two subgroups. Therefore, patients were divided into the following four groups: Group Ia, total or near total therapeutic effect; Group Ib, subjectively more than 50% therapeutic effect but less than total or near total; Group IIa, less than 50% therapeutic effect, but effect evident; and Group IIb, no therapeutic effect. Pathologic response rate was defined as a ratio of responders to total number of tumors.

Immunohistochemical analysis

The Immunohistochemical analyses were carried out using the method previously described [16]. Immunohistochemical analyses were performed on formalin-fixed, paraffin-embedded sections of surgical specimen. The slides were deparaffinized in xylene and rehydrated in gradient ethanol solutions. Endogenous peroxidase was blocked with 0.3% H_2O_2 in methanol for 5 minutes. The slides were immersed in 10 mM citric buffer (pH 6.0) with heating for 15 minutes for antigen retrieval. Nonspecific binding was blocked by preincubation with 10% fetal calf serum in PBS with 0.01% sodium azide, and the slides were incubated in a humid chamber for 1 hour with antibody against Lin28 (rabbit polyclonal, H-44, Santa Cruz; 1:100). Washing three times in PBS, the slides were incubated with the EnVision-HRP complex (undiluted, DAKO) for 60 minutes. The slides were visualized with diaminobenzidine (DAKO Corp.) and then counterstained with hematoxylin. For substitute negative controls, the primary antibody was replaced with phosphate buffered saline. Positive control was breast cancer tissues known to exhibit high expression level of Lin28.

The expression of the antibodies was assessed semi quantitatively by estimating the percent-

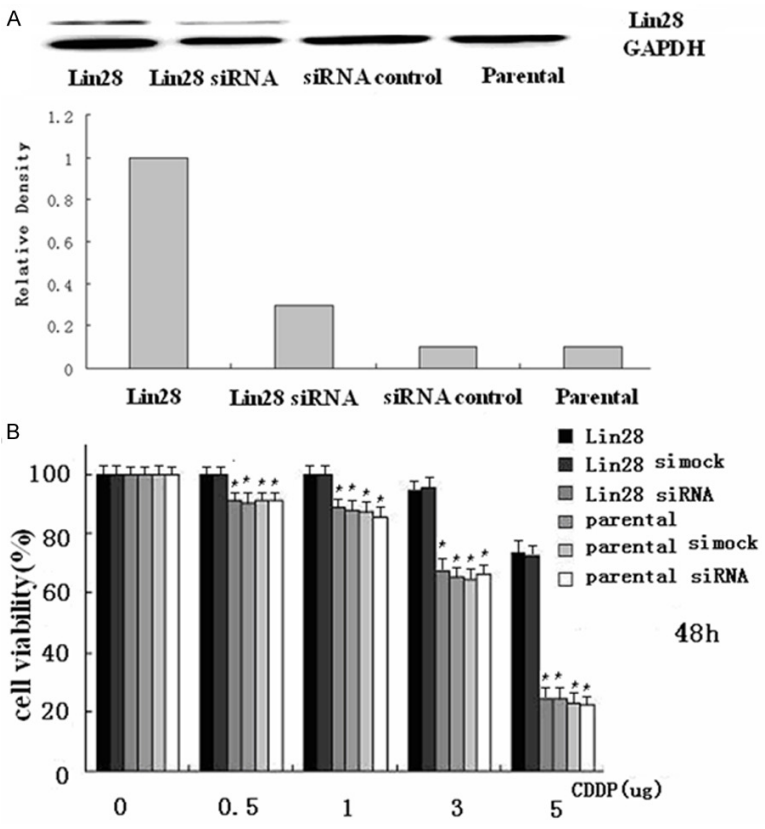

Figure 2. Lin28 depletion by microRNA chemosensitized SGC7901/Lin28 cells to cisplatin. A. Knockdown of Lin28 expression in SGC7901/Lin28 cells. Whole cellular protein extracts of SGC7901 cells transfected with empty vector or pcDNA3.1$^+$/Lin28 were subjected to Western blot analysis using ant-Lin28 antibody. B. Knocking down of Lin28 by Lin28 siRNA reverses cisplatin resistance in highly Lin28 expression SGC7901/Lin28 cell line. Parental and siRNA control transfected SGC7901 were used as controls. MTT assays. Cells cultured in 96-well and viability was evaluated by MTT assays after both 48 of cisplatin treatment. Cells treated with PBS were used as a control. Columns, mean of three independent experiments; *P < 0.001, when compared with the group treated with cisplatin in the SGC7901/mock.

age of tumor cells with positive cytoplasm staining on whole tumor slides. All the slides were examined and scored independently by two experienced pathologists to avoid subjective biases. Each slide was examined in its entirety under a light microscope, and initially a proportion score was assigned, which represented the estimated proportion of positive tumor cells (0, none; 1, 0~10%; 2, 10%~50%; 3, 50%~100%). Next, an intensity score was assigned, which represented the average intensity of the positive tumor cells (0, none; 1, weak; 2, intermediate; and 3, strong). The proportion and intensity scores were then multiply obtain a total score, which ranged from 0 to 9, and Lin28 protein positive expression was defined as total score ≥ 2.

Statistical analysis

All statistical analyses were conducted using the statistical program SPSS 15.0 for windows (SPSS, Chicago, IL, USA). All of the experiments were performed three times with triplicate samples. Analysis of variance and Student's t test were used to compare the values of the test and control samples. The accepted level of significance was set as $P < 0.05$.

Results

Stable transfection and expression of Lin28 in SGC7901 cells

The gastric carcinoma SGC7901 cell line has been used as a well-characterized cell model in evaluation of drug resistance in gastric carcinoma [17]. To investigate the possible influence of Lin28 status on the sensitivity of gastric carcinoma cells to cisplatin, we restored expression of Lin28 in SGC7901 cells through stable transfection of Lin28 expression vector (pcDNA3.1$^+$-Lin28). Western blotting confirmed the expression of exogenous Lin28 in clones' transfected with pcDNA3.1$^+$-Lin28 cDNA. As shown in **Figure 1A**, the SGC7901 cell line transfected with empty vector does not express Lin28 (lane 6, SGC7901/mock), individual Lin28-transfected clones 2, 3 and 5 (lanes 2, 3 and 5, SGC7901/Lin28/c2, SGC7901/Lin28/c3 and SGC7901/Lin28/c5), express high levels of exogenous Lin28. Clone 1 and 4 express low levels of exogenous Lin28 protein (lane 1 and 4) and was not used for further studies.

SGC7901 cells expressing Lin28 exhibit reduced sensitivity to cisplatin

The MTT assays to determine whether the expression of Lin28 would affect the sensitivity of SGC7901 cells with or without Lin28 to cis-

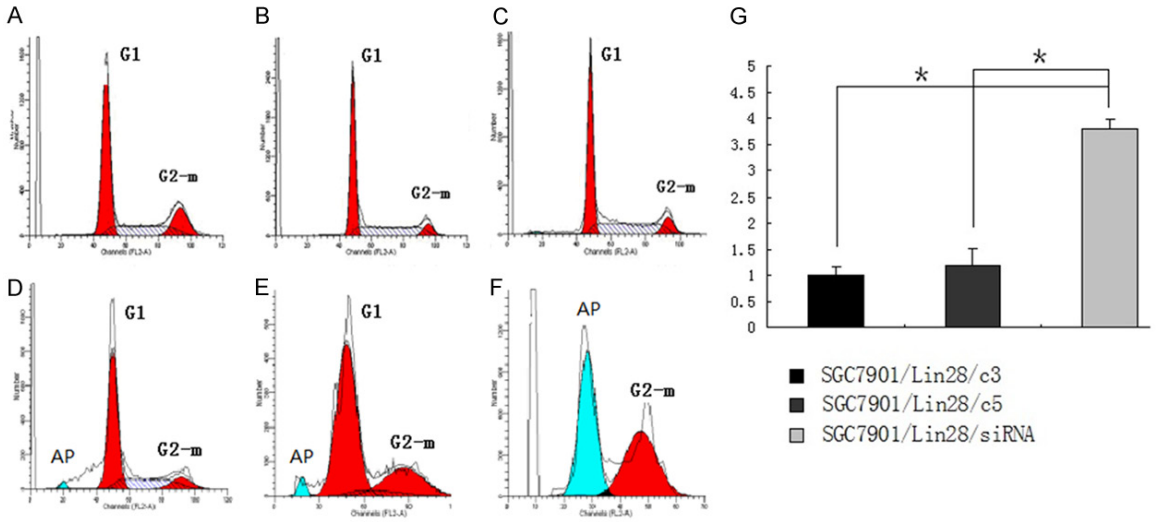

Figure 3. Flow cytometric analyses of cell cycle distribution and apoptosis. A. The cell cycle distribution of SGC7901/parental cell lines. B. The cell cycle distribution of SGC7901/Lin28/siRNA cell lines. C. The cell cycle distribution of SGC7901/Lin28/c3 cell lines. There were no significant in three groups ($P > 0.05$). D. The cell cycle distribution and apoptosis of SGC7901/parental cell lines after 48 h of cisplatin treatment. E. The cell cycle distribution and apoptosis of SGC7901/Lin28/siRNA cell lines after 48 h of cisplatin treatment. F. The cell cycle distribution and apoptosis of SGC7901/Lin28/c3 cell lines after 48 h of cisplatin treatment. Knocking down of Lin28 by Lin28 siRNA reverses cisplatin resistance in SGC7901/Lin28/c3 cell line. G. The percentages of cisplatin induce apoptosis are demonstrated as shown.

platin. Briefly, the cells transfected with empty vector (SGC7901/mock), and three selected Lin28-positive individual clones (SGC7901/Lin28/c2, SGC7901/Lin28/c3 and SGC7901/Lin28/c5) were treated with 0, 0.5, 1, 3 and 5 μg/ml cisplatin. MTT assays were done after cells were exposed to cisplatin for 48 and 72 h, respectively. As depicted in **Figure 1B**, the cell with Lin28-transfected viabilities after cisplatin treatment were much higher in all cell lines transfected with Lin28 than that of the cell line transfected with vector only ($P < 0.05$ or $P < 0.001$; see **Figure 1B**). These results indicate that, the overall cytotoxic effects of cisplatin were significantly reduced in the SGC7901 cells expressing Lin28.

To further confirm the direct correlation between Lin28 and the resistance to cisplatin in gastric carcinoma cells, we knocked down Lin28 expression by Lin28 siRNA in SGC7901 (SGC7901/Lin28/C3) carcinoma cells with Lin28 highly expression, and determined their sensitivity to cisplatin. Moreover, we showed that the treatment with siRNA-Lin28 significantly down-regulated the protein levels of Lin28 in SGC7901/Lin28/c3 cells (**Figure 2A**). The SGC7901 cells with or without Lin28 were treated with 0, 0.5, 1, 3 and 5 μg/ml cisplatin. MTT assays were done to evaluate the cell viability and the sensitivity to cisplatin-induced apoptosis. As indicated in **Figure 2B**, pretreatment with siRNA-Lin28 completely prevents Lin28-induced resistance to cisplatin. In parental SGC7901 and SGC7901/mock cell lines, SGC7901/Lin28/siRNA cells had no significant effect on overall cytotoxicity.

Lin28 transfection inhibits cisplatin-induced apoptosis

Cisplatin has been shown to induce apoptosis in gastric carcinoma cells, so we next determined the effect of Lin28 transfection on cisplatin-induced apoptosis. We compared the influence of Lin28 on the ability of cisplatin to induce tumor cells apoptosis in SGC7901 cells. From the flow cytometry results presented in **Figure 3**, we observed that, after 48 h of cisplatin treatment, the majority of tumor cells were accumulated at G2-M phase in SGC7901/Lin28/c3, SGC7901/Lin28/siRNA and SGC7901/parental cell lines. The expression of Lin28 cells after 48 h of cisplatin treatment significantly decreases the population of apoptotic population of tumor cells in the SGC7901/Lin28/c3 cell lines compared with SGC7901/

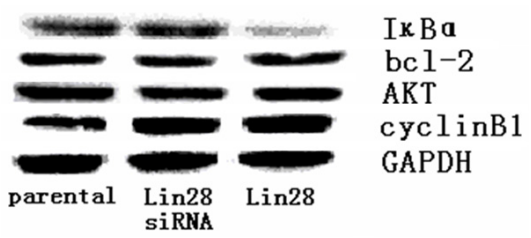

Figure 4. Western blot analyses for the IκBα, bcl-2, AKT, and cyclin B1 proteins. Cells were treated with 3 ug/mL cisplatin. Whole-cell proteins were extracted from cells after 48 h of cisplatin treatment. Equal amounts (40 Ag/lane) of cellular protein were fractionated on 10% to 12% SDS-polyacrylamide gel and transferred to polyvinylidene difluoride membranes, followed by immunoblotting with anti-IκBα, bcl-2, AKT, and cyclin B1 antibodies. GAPDH protein was blotted as a control.

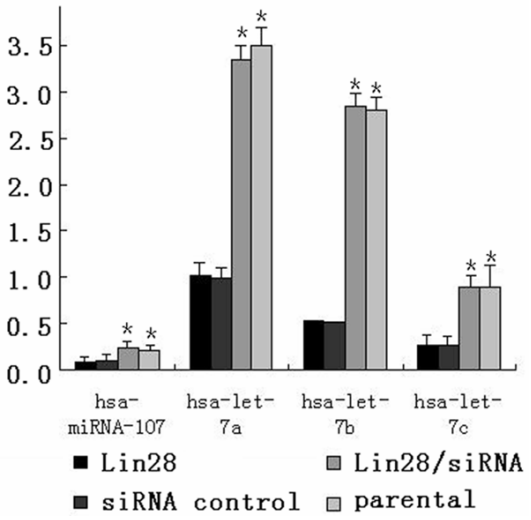

Figure 5. Lin28 downregulates Let-7 miRNA expression. Expression of Let-7a, Let-7b and Let-7c miRNAs in SGC7901 cells stably expressing Lin28 was examined by qPCR. The empty vector transfected SGC7901 cells and SGC7901 parental cells were used as controls.

Lin28/siRNA and SGC7901/parental cell lines. Further, we did cytospin assays and confirmed that Lin28 expression has effect on cisplatin induce apoptosis.

Lin28 Inhibits Let-7 miRNA and IκBα

To further investigate the mechanism of Lin28-induced cisplatin resistance, we measured the expression of some key genes and of Let-7 miRNA in the SGC7901 cells that stably expressed Lin28. Western blotting analysis showed that IκBα was significantly decreased in the cancer cells stably expressing Lin28 compared to the mock controls, whereas bcl-2, cyclin B1 and AKT expression did not change (**Figure 4**). Real-time PCR analysis also showed that Let-7a, Let-7b and Let-7c miRNAs were dramatically decreased in the cancer cells stably expressing Lin28 compared to the mock controls (**Figure 5**).

Lin28 Associated with chemoresistance in gastric cancer patients

The pathologic responses were evaluated in all 21 gastric carcinoma patients at the end preoperative chemotherapy administration. According to the criteria defined above, 1 of 21 (4.8%) patients had Ia (total/near total therapeutic effect); 13 of 21 (61.9%) patients had Ib (subjectively > 50% therapeutic effect but < total/near total therapeutic effect); 7 of 21 (33.3%) patients had IIa (< 50% therapeutic effect). For further analysis, patients were divided into two categories based on the response to treatment: responders (Ia + Ib) and nonresponders (IIa + IIb) (data not show).

The histological effects of preoperative chemotherapy in primary gastric carcinoma with different Lin28 status were evaluated by comparing gastroscopy samples and surgical samples taken before and after chemotherapy. Tumor samples were fixed in formalin for hematoxylin and eosin (H&E) staining and immunohistochemical staining with anti-Lin28 antibody using standard protocols. Various pathological changes were observed after preoperative chemotherapy, which mainly included coagulative necrosis of tumor tissue, fibrosis/hyalinization, as well as mixed inflammatory infiltrate. Chemotherapy could also induce cytological changes in tumor cells in cytoplasm and/or nuclear. For example, the cytoplasm of tumor cells was either intensely eosinophilic or clear with a vacuolated or foamy appearance. Enlarged or bizarre nuclear with clumped chromatin may be recognized. Furthermore, the volume of residual tumor varied based on different chemotherapeutic effects. In our study, most of Lin28-negative gastric carcinoma tissues showed significant better pathologic response to chemotherapy than Lin28-positive tumors (data not show). Lin28 expression is significantly associated with decreased responders in patients with gastric carcinoma (P= 0.024).

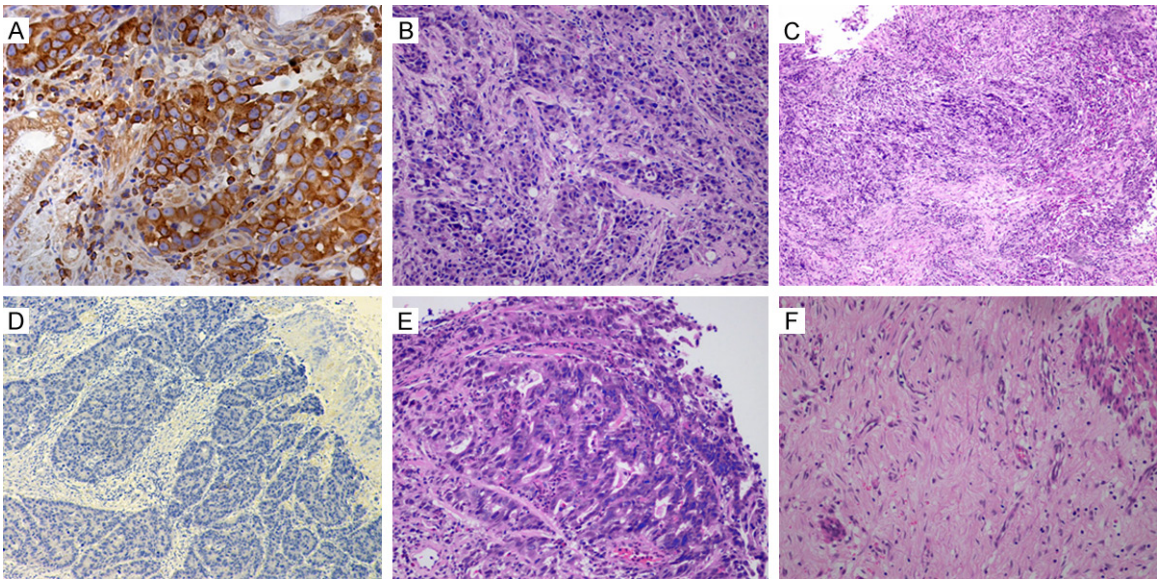

Figure 6. Immunohistochemical staining with Lin28 and H&E staining. Stainings were performed on formalin-fixed, paraffin-embedded tissue sections of invasive gastric carcinoma, and photographed before and after preoperative chemotherapy, by using a Zeiss Axioskop 40 Microscope and a Nikon E4500 camera. A. Lin28 expression is strong positive in representative slide. B. H&E stained section of gastric carcinoma gastroscope specimens with Lin28 positive expression (before neoadjuvant chemotherapy). C. H&E stained section of gastric carcinoma resected specimens with Lin28 positive expression (after neoadjuvant chemotherapy). D. Lin28 expression is negative in representative slide. E. H&E stained section of gastric carcinoma gastroscope specimens with Lin28 negative expression (before neoadjuvant chemotherapy). F. H&E stained section of gastric carcinoma resected specimens with Lin28 negative expression (after neoadjuvant chemotherapy). Data are representative of at least three separate experiments. Magnification: ×100.

As shown in **Figure 6**, there were still many survival tumor cells with Lin28 positively after the preoperative chemotherapy Lin28-positive primary gastric carcinoma. However, the Lin28-negative primary tumor exhibited significantly improved pathologic response to chemotherapy, which was demonstrated by the dramatically reduced volume of residual tumor, as well as the remarkable fibrosis and hyalinization of tissue after the treatment.

Discussion

Drug resistance is one of the major obstacles limiting the success of gastric carcinoma chemotherapy. In this field, inherent or acquired chemotherapy resistance, which can include development of resistance to multiple drugs, is a frequent phenomenon in gastric carcinoma cells. Forty percent of gastric carcinoma patients with surgery and 80% of gastric carcinoma patients with unresectable disease have poor response to chemotherapy [1, 18]. Several mechanisms of drug resistance have been examined. Overexpression of a membrane efflux transporter, P-glycoprotein (P-gp), overexpression of multidrug resistance (MDR)-associated protein, changes in topoisomerase II activity , modifications in glutathione S-transferase, and altered expression of apoptosis-associated protein Bcl-2 and tumor suppressor protein p53 [19-21]. Lin28 status, although lesser known, have also been studied in connection with MDR.

Lin28 was confirmed ability to facilitate the reprogramming of human somatic cells to induced pluripotent stem (iPS) cells [6]. More recently, Lin28 was reported to play important roles in stem cells [22, 23]. Nevertheless, it remains unknown how Lin28 functions in the development and progression of gastric carcinoma. The important roles of Lin28 in stem cells and various cancers prompted us to investigate the level of Lin28 expression in human gastric carcinoma and the effects of its alteration. Our previous studies found that the positive expression of Lin28 protein in gastric carcinoma patients were significantly associated with poor prognosis. Furthermore, a multivariate analysis showed that positive Lin28 protein expression was an independent prognostic fac-

tor associated with overall survival [24]. This finding implies that the expression Lin28 might mediate gastric carcinoma cells death.

To identification the role of Lin28 in gastric carcinoma chemotherapy, we stable transfection and expression of Lin28 in SGC7901 cells result in reduced sensitivity of SGC7901 cells to ciaplatin, particularly to ciaplatin-induced apoptotic cell death. In the present study, we did a series of assays to examine the mechanism of the Lin28 mediated tumor cells resistance to cisplatin. By cytospin assay and flow cytometry assays, we determined that Lin28 could significantly interfere with the ability of cisplatin to induce apoptosis in SGC7901 cells expressing exogenous Lin28. These findings suggest that Lin28 status might play an important role in determining the sensitivity of gastric carcinoma to cisplatin and possibly other chemotherapeutic agents.

To investigate the possible molecular mechanisms by which Lin28 mediates resistance to cisplatin, we have examined several genes or regulatory proteins that may contribute to cisplatin activity in inducing cell cycle arrest and apoptosis. Our results demonstrated that ectopic expression of Lin28 inhibited gastric carcinoma cells sensitivity to cisplatin treatment and that Lin28-mediated anti-cisplatin activity is caused by inhibition of Let-7 miRNA and IκBα genes expression. The role of the Let-7 gene in cancer cell chemosensitivity has been studied by several groups, and accumulating evidence has shown that low Let-7 expression promotes cancer cell invasion and metastasis is associated with low chemosensitivity in esophageal cancer, pancreatic cancer cells, hepatocellular carcinoma cells and oral cancer cells [25-27]. Although the role of Let-7 in gastric carcinoma chemosensitivity was not well characterized previously, Let-7 had been shown associated with tumor invasion and metastasis in human gastric carcinoma [28].

Several biological factors, including P-gp, multidrug resistance (MDR)-associated protein, bcl-2, and TOP2A, have been reported to be associated with chemosensitivity after sequential chemotherapy in gastric carcinoma. In our study, most of Lin28-negative gastric carcinoma tissues showed significant better pathologic response to chemotherapy than Lin28-positive tumors. Lin28 expression is significantly associated with decreased responders in patients with gastric cancer. The clinical significance of identification of this marker for the prediction of response to chemotherapy therefore seems to deserve further investigation.

In summary, this study shown that Lin28 protein expression could be used as a potential molecular marker to predict chemosensitivity in gastric carcinoma patients. The present study has investigated whether the differential sensitivity of gastric carcinoma cells to cisplatin might be associated with the status of Lin28. Through stable transfection of Lin28 expression vector into SGC7901 cells, we found that, the expression of Lin28 clearly decreased the sensitivity of SGC7901/Lin28 cells to cisplatin-induced cell-killing activity and apoptosis. The clinical trials observations have suggested Lin28 expression is significantly associated with decreased responders in patients with gastric carcinoma. Therefore future investigation on the precise roles of Lin28 in gastric carcinoma will provide new insights, which will contribute to improve diagnosis and treatment.

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Disclosure of conflict of interest

None.

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